ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Procedia Engineering 10 (2011) 2399–2404

Engineering Procedia

ICM11

Surface Characteristics and Cell Proliferation of Mechanical Sandblasted Ti-30Ta-xNb Surface

W. G. Kim[1], K. H. Ahn[2], Y. H. Jeong[1], M. S. Vang[2], and H. C. Choe[1] *

[1]*Department of Dental Materials, School of Dentistry, Chosun University, Gwangju 501-759, Korea*

[2]*Department of Prosthodontics, School of Dentistry, Chonnam National University, Gwangju 500-757, Korea*

Abstract

In this study, surface characteristics and cell proliferation of mechanical sandblasted Ti-30Ta-xNb surface have been investigated. Samples with three different surface preparations were used: 0.3um polished, 50um and 100um HA sandblasted. Cell culture on the specimen surface was performed with mouse osteoblast-like cells MC3T3-E1 for 2 days. Average surface roughness and surface morphology of specimens were observed using surface roughness tester, and field-emission scanning electron microscopy. The corrosion resistance was measured using electrochemical impedance spectroscopy and potentiodynamic test at 0.9% NaCl solution at 36.5°C. The average surface roughness of Ti-30Ta-xNb (x=5, 10, 15wt%) alloys increased with increasing blasting particle size and Nb content. Cell adhered well on the rough surface of Ti-30Ta-xNb (x=5, 10, 15wt%) alloys. Cell growth increased as Nb content increased. From the electrochemical test, Ti-30Ta-xNb (x=5, 10, 15wt%) alloys blasted by 100um HA particles showed lower dissolution rate than that by 50um HA particles, especially, Ti-30Ta-15Nb alloy showed lower dissolution rate.

Selection and peer-review under responsibility of ICM11

Keywords: Mechanical sandblasting; cell proliferation; Ti-30Nb-xTa alloy; electrochemical behavior; surface roughness

1. Introduction

Since Ti-6Al-4V alloy which has α+β mixed phase has high intensity, it is usually used for implant, bone plates and clamp, etc but since the element like V causes the cytotoxicity, Semlitsch *et al.*[1] developed the biomaterials with excellent biocompatibility, mechanical properties, corrosion and abrasion resistance by developing Ti-6Al-7Nb and Ti-5Al-2.5Fe alloy adding Nb or Fe instead of V. However, as Al has known as the cause of Alzheimer [2], the study of replacing into Al element has been progressed. Due to the difference of elastic modulus between bone (20 GPa) and Ti-6Al-4V alloy (106 GPa), stress shielding is occurred and high stress is delivered to adjacent bone so biocompatibility is reduced greatly or clinical failure takes place. This phenomenon is remained as the limitations of metallic biomaterials, and thus to improve this problem, it has been focused on the study of developing β-type Ti alloy which has the similar elastic modulus with the bone. According to Zhou *et al.* [3], since Ti-Ta alloy has high intensity as well as low elastic modulus, they reported that it has high possibility to replace as biomaterials and Ti-

* Corresponding author. Tel.: +82-62-230-6896; fax: +82-62-230-6896.
E-mail address: hcchoe@chosun.ac.kr.

1877-7058 © 2011 Published by Elsevier Ltd. Open access under CC BY-NC-ND license.
Selection and peer-review under responsibility of ICM11 doi:10.1016/j.proeng.2011.04.395

Nb allow is also known as having low elastic modulus. Like this, by adding Nb or Ta element to Ti, the alloy with low elastic modulus can be obtained since these elements are β-stabilizer [4]. According to the report studied previously, Hf and Zr increase the elastic modulus and Ta and Nb reduces the elastic modulus. There are several mechanisms and as Ta and Nb content are increased, it expands the unit cell of crystalline structure and the static modulus is decreased due to this. According to Buser *et al.*[5], the affinity between bone and implant is increased depending on the surface roughness, the excellent of bonding between bone tissues in the implant with surface treated was reported. These studies are methods of evaluating the growth of cell or the degree of attachment that surface roughness affects on the osseointegration and in case of the cell growing on the surface, the study about the effect of osseointegration due to metal ion dissolution has been insignificant. From this, the study about how the cell growth happens depending on the surface roughness and how this affects on the metal ion dissolution through the electrochemical evaluation on the metal surface where the cell is cultured is needed.

In this study, RBM was treated using HA particles of different sizes on the surface of alloy to grant the roughness after preparing the Ti-Ta-Nb alloy which is β-type Ti alloy, and by incubating the MC3T3-E1 osteoblast cells, the electrochemical characteristics on the surface according to this were to find out.

2. Experimental

To prepare Ti-30Ta-xNb (x=5, 10, 15wt%) alloy on the basis of Ti-30Ta alloy which has low elastic modulus, the treatment was performed after preparing the alloy with high intensity and low elastic modulus by changing the content of Nb to Ti-30Ta. The Ti-30Ta-xNb (x=5, 10, 15wt%) alloy was prepared using vacuum arc furnace and by measuring the composition of Nb and Ta as 30 wt.% respectively, For homogenization, the prepared specimen was fast-cooling in the water at 0℃ after maintaining for 24 hours at 1000℃ in Ar atmosphere inside the electric furnace (Model KDF-S70.1000 DENKEN, JAPAN).

The specimens were RBM (resorbable blasting media) treated using HA (hydroxyapatite) particle of 50 ㎛ and 100 ㎛ on the surface of specimen, respectively after ultrasonic cleaning for 10 min. The blast processing which is air blow method was performed for 2 min with 6 kg/cm^2 of pressure to the surface of implant alloys. After blasting, the average surface roughness (R_a) was measured more than 10 times with surface roughness tester (DSF-1000, Kosaka, Japan).

To observe the growth behavior of the cell on the surface assigned with the surface roughness, MC3T3-E1 osteoblasts which are the cell line extracted from cranial bone was cultured for 48 hours at 37℃ with 4 x 10^4 cells/cm^2 using α-MEM (alpha modified Eagle's minimum essential medium) added with 10% FBS (fetal bovine serum). After cultivating, it was stabilized for 20 min with 70% alcohol after washing with phosphate buffer solution (PBS). After that, it was fixed with 2.5% glutaraldehyde and washed with the same buffer. After finishing the washing, the post-fixation was done at $HgCl_2$ supersaturated solution added with 1% OsO_4 and it was dehydrated with ethanol. To study the cell adhesion, the cell count was investigated with hemacytometer by extracting the cells after cultivating the cells with the same method of cell culture method in the above.

To investigate the general corrosion characteristics of alloys prepared, the potentiodynamic test was performed with 1.66 mV/sec of scanning speed in 0.9% NaCl. By stabilizing the surface of specimen by artificially reducing for 10 min under the cathodic potential of -1500mV and at the same time stirring by injecting Ar gas, the impurities, oxide and dissolved oxygen on the surface of specimen were removed. The polarization test was performed by adding the potential from -1500 mV to +2000 mV with the same condition for each specimen, and the corrosion potential, corrosion current density, passive current density and pitting potential were measured.

AC impedance test was measured in 0.9% NaCl of 36.5 ± 1℃ which is the same as the electrochemical potetiodynamic test. By obtaining Nyquist plot, Bode plot and Bode-phase plot with the frequency range used in the measurement from 10 mHz of low frequency to 100 kHz of high frequency, the electrochemical behavior was studied by investigating the polarization resistance (R_p) values and the resistance of the solution (R_Ω) from this.

3. Results and discussion

3.1. Microstructure of Ti-30Ta-xZr alloys

This study is for investigating the electrochemical characteristics by cell culture after performing RBM treatment to prepared alloys, and when looking at the structure of previously developed Ti-30Ta-xNb (x=5, 10, 15wt%) alloy, as Nb increases from 5wt% to 15wt% Nb in the homogenized structure, in α +β phase structure, α phase was significantly decreased and β phase structure was more prominently formed, it is thought that this is because Nb was acting as a stabilizing element of β phase structure[6]. This can be checked from the result of XRD, from the x-ray diffraction peak (Fig. 1) when Nb content was added 5wt%, at about 38° β phase peak is observed, and at about 40°and 70°, α phase peak was observed so it is confirmed that the mixed phase of α +βis existed at low content of Nb and as the content of Nb is increased, the transition from α phase to β phase takes place. These results are thought to be reduced by α phase peak when Nb was added to Ti-30Ta which has α+β phase structure because Nb is the β phase stabilizing element.

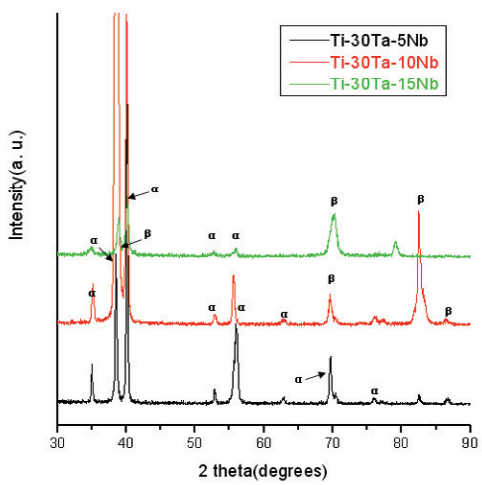

Fig. 1. XRD patterns of Ti-30Ta-xNb alloys.

3.2. Surface roughness and cell culture of Ti-30Ta-xNb alloys

To give the roughness to the surface of Ti-30Ta-xNb(x=5, 10, 15wt%) alloy, the roughness is checked by RBM treatment, the roughness is significantly increased compared to the specimen with polishing treatment. From Fig. 2 and 3, the appearance of surface roughness can be seen clearly, in case of RBM treatment, as HA particle size is increased from 50 µm to 100 µm, the surface roughness is significantly increased. This is because as each size of particles are collided to the surface, the appearance of particle leaves the mark on the surface, and at this time, it is not only affected by the size of particle, but also the particle injection pressure significantly. In this study, since the injection pressure was set as 6 kg/cm^2, the size of particle and the ductility of the base material have influence on the roughness formation directly. As the content of Nb is increased, the base material is changed to β-phase and the crystal grain is enhanced, so it has the characteristics of ductility[7]. This can be seen from the marks formed on the surface, and when looking at the surface pictures of Ti-30Ta-5Nb andTi-30Ta-15Nb, there is no big difference as a whole, but if looking at closely, the deep and ductile marks were formed in the surface of Ti-30Ta-15Nb. In other words, when comparing Fig. 2(c) with Fig 3(c), even though it was treated with blasting on the surface with the same size of HA particles, in Fig. 3(c), the marks that the particle was embedded are wide while Fig. 2(c) was appeared as narrow so this could be confirmed. This can be thought that as Nb is added, the workability is easier and the elastic modulus can be reduced. For blasting with 50 µm and 100 µm, in terms of roughness, the surface feature is significantly increased in case of blasting with 50 µm (Table 1) but if the surface feature was observed from the picture, in case of 50 µm blasting, while the area that can cause the electrochemical reaction is increased largely by colliding the small particles on the surface, in case of 100 µm blasting, the area which can react electrochemically is decreased.

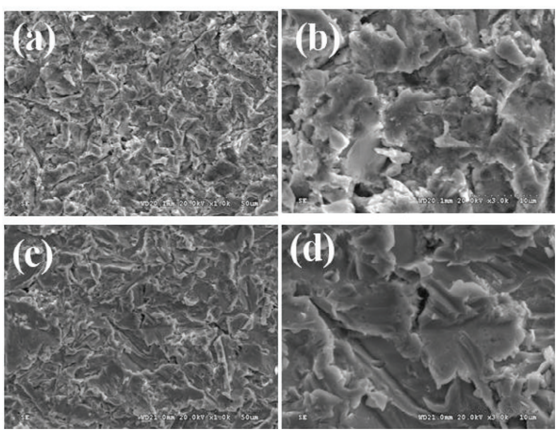

Fig. 2. FE-SEM micrographs showing the surface of sandblasted Ti-30Ta-5Nb alloys. (a), (b) 50 µm sandblasted (c), (d) 100 µm sandblasted

Table 1. Average surface roughness values(R_a:µm) of surface modified Ti

Sample \ Values	Ti-30Ta-5Nb	Ti-30Ta-10Nb	Ti-30Ta-15Nb
polished	0.105	0.109	0.112
50 µm sandblasted	0.982	1.113	1.171
100 µm sandblasted	1.594	1.608	1.685

alloys

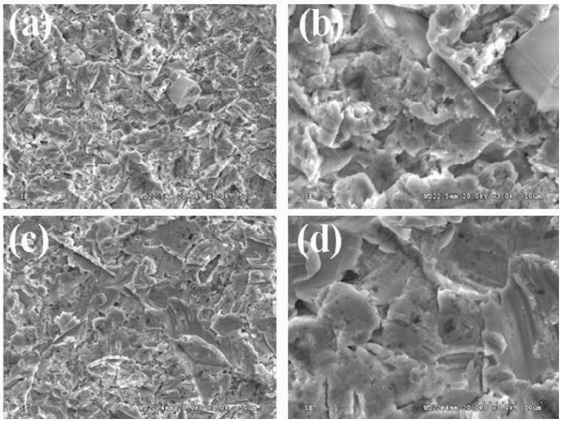

Fig. 3. FE-SEM micrographs showing the surface of sandblasted Ti-30Ta-15Nb alloys. (a), (b) 50 μm sandblasted (c), (d) 100 μm sandblasted

The grant of surface roughness is to enhance the osseointegration by stabilizing the cell growth. Since these osseointegrations are important for surface roughness as well as metal ion dissolution contained in the alloy elements, depending on the alloy elements and surface roughness, it is necessary to investigate the ion dissolution behavior in the surface of cell culture.

As a result of observing the surface with SEM by cultivating MC3T3-E1 osteoblasts on the surface of alloy after RBM treatment using HA particle on the surface of Ti-30Ta-xNb (x=5, 10, 15wt%) alloy, cells grew irregular form in every specimens, and in case of specimen treated with grinding, the long cytoplasmic prolongation and various cytoplasmic microvilli were observed in various direction (Fig. 4, 5). From the reports of previous researchers, cells are proliferated a lot in the smooth surface, but in rough surface, cells are not proliferated but differentiated significantly, and in smooth surface, there are disadvantage that adhesion is weak[8-9]. In this study, similar to the previous studies, cells were proliferated in various directs in the smooth surface, and in rough surface, cells were significantly differentiated. This can be confirmed from the values measuring cell growth and adhesion. The surface roughness showed the smoothest surface after grinding treatment, and in case of blasting treatment, as the particle size of HA was increased, the surface was roughened. Therefore, for cell growth the large cells is mainly shown in rough specimen depending on the surface roughness, and in case of grinding, cells are covered the whole surface. Therefore, since cell proliferation is increased in the specimen with the smooth surface, but the degree of surface adhesion is reduced, and the effect of increase in cell adhesion can be achieved in the rough surface, the proper surface roughness should be given. In other words, to enhance the osseointegration, the surface roughness which is combined with small range of roughness and large range of roughness is needed. In general, the average surface roughness of dental implant is known as 1.0 ~ 1.5 μm [10].

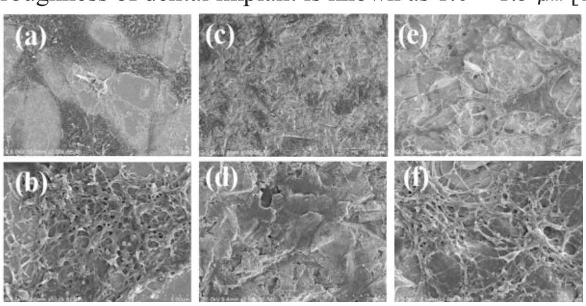

Fig 4. SEM micrographs showing the surface of MC3T3 E-1 cell cultured Ti-30Ta-5Nb alloys. (a),(b) polished (c),(d) 50 μm sandblasted (e),(f) 100 μm sandblasted

It is known that the surface roughness is significantly affected on the electrochemical characteristics of implant surface and typically as the area where the electrochemical reaction of surface can occur is increased, the electrical reaction is activated and the corrosion resistance is reduced greatly[11]. After cultivating MC3T3 E-1 cell, depending on the surface roughness, the specimen treated with grinding has high corrosion potential and low current density compared to the specimen treated with RBM, so it has better corrosion resistance, and depending on the particle size of RBM treatment, in case of 100 μm particle RBM treatment has better corrosion resistance than in case of 50 μm particle RBM treatment and this is thought to have more spaces provided for electrochemical reactions.

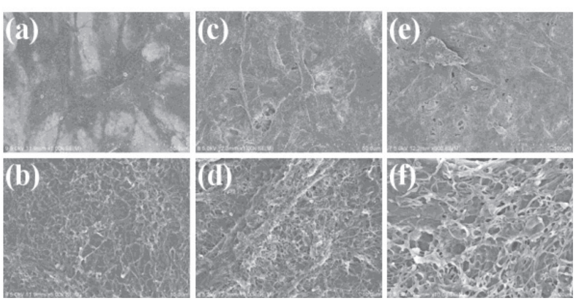

Fig 5. SEM micrographs showing the surface of MC3T3 E-1 cell cultured Ti-30Ta-15Nb alloys. (a),(b) polished (c),(d) 50 μm sandblasted (e),(f) 100 μm sandblasted

When looking at the corrosion characteristics depending on alloy compositions, from the result of electrochemical potentiodynamic curve (Fig. 6) of Ti-30Ta-xNb (x=5, 10, 15wt%) alloy before blasting, it is showing that overall a wide passive film was formed on the passive region and as the content of Nb is increased, the corrosion resistance is significantly increased. This is thought that because the grain boundary area is decreased due to the particle growth and Nb and Ta are formed the oxide[3] such as TiO_2, Nb_2O_5, Ta_2O_5 with Ti on the surface.

3.3. Electrochemical behavior of cell cultured Ti-30Ta-xNb alloys

After cultivating MC3T3 E-1 cells, from the result of electrochemical potentiodynamic curve of Ti-30Ta-xNb (x=5, 10, 15wt%) alloy, there is the instable current change curve at the passive region, and this is thought that because it has effect of inhibiting the current flow by grown cells on the surface. This phenomenon is considered to show the corrosion inhibition effect by interference the penetration of Cl^- ion presented inside the 0.9% NaCl by MC3T3-E1 cells. In the polarization curve, anode reaction increases the current, while formation of passive film reduces the current by interference the activation surface of electrode. It is reported that due to these two spontaneous phenomena, the irregular changes of current density is occurred[12]. There are several causes for irregularity of anodic polarization curves, but it is characterized by an irregular curve in solution existed with halide ions that can occur the formula. These irregular changes of current density has appeared over a much wider range of area in case of cultivating MC3T3-E1 cells than not cultivating cells and it is considered that it has effects on the electrochemical characteristics by appearing the irregularity of current density which can appear on insulators such as ceramic by existing cells on the surface (Fig. 6(b)). When the potential increases after passing this unstable area, the detachment of cells from the surface of the specimen is occurred, the surface is starting to become stable again by finding the characteristics of the conductor, the passive region appears clearly on the polarization curves, and the same trend begins to show with the case without cell culture.

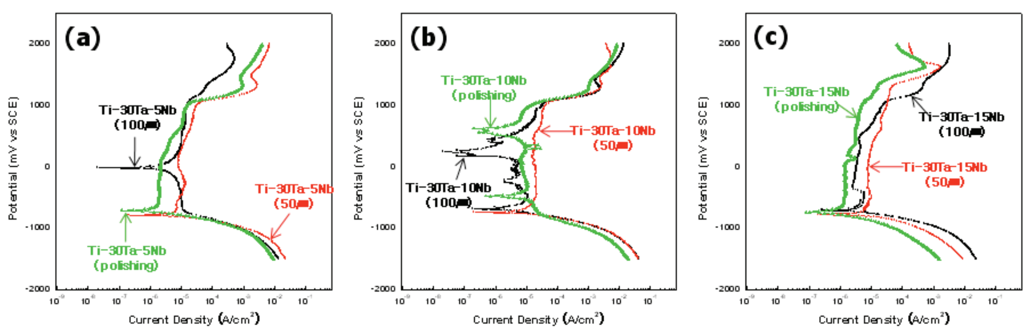

Fig. 6 . Anodic polarization curves of Ti-30Ta-xNb alloys with various surface treatment in 0.9% NaCl solution at 36.5±1℃ after cell culture. (a) Ti-30Ta-5Nb (b) Ti-30Ta-10Nb (c) Ti-30Ta-15Nb

As a result of measuring the impedance from the surface of specimen treated with grinding, 50 ㎛ and 100 ㎛ RBM after cultivating cells, the impedance is significantly increased at low frequency in case of Nyquist plot, and in case of 15wt% Nb added, with the unlimited increase of the impedance, also it shows the excellence in corrosion resistance[13]. In case of RBM treatment, the polarization resistance value appeared as 10^4Ω㎠, so the corrosion resistance is significantly reduced compared to the case with grinding treatment. Especially, in case of cell culture with 50 ㎛ RBM treatment the polarization resistance value is reduced and corrosion resistance is reduced compared to the case of cell culture with 100 ㎛ RBM treatment. As considered earlier, this is because there are many areas formed for electrochemical reactions occurring in the surface.

Regarding the characteristics of the Bode-phase plot, in all specimens, it can be observed that the phase angle is falling from high frequency toward 0° and increases again. The impedance in this high frequency area is occurred due to the resistance (R_Ω) of solution. It can be observed that the phase angle is falling toward the lower values in the middle frequency and low frequency area. This is considered that the impedance is dominated by the resistance of surface film. Also, throughout the wide frequency area, phase angle is existed as constant phase close to 90° and this causes the capacity reaction from all specimens and this phenomenon is occurred because the passive layer that existed in the surface is formed. From these results specimens with cell culture and grinding treatment have excellent electrochemical characteristics.

4. Conclusion

As a result of analyzing XRD phase in Ti-30Ta-xNb alloy, α+β phase was appeared as a whole and as the content of Nb was increased, the change from α phase to β phase was happened. The surface roughness was increased as the blasting particle was larger, and as the Nb content increased, it had tendency to increase slightly. As a result of cell culture, as the surface roughness was increased, the cell adhesion was good and as the Nb content was increased, the cell growth was increased. As a result of electrochemical test before and after cell growth, in case of blasting with 50 ㎛ has higher ion dissolution rate than blasting with 100 ㎛ and when 15wt% of Nb was added, the dissolution rate of ion was lower. Consequently, in case of 50 ㎛ particle RBM of Ti-30Ta-xNb (x=5, 10, 15wt%) alloy has decreased corrosion resistance more than in case of 100 ㎛ particle RBM and the cell growth is largely increased as the surface roughness is increased. Also, in case of cell culture in the surface, it has influence on the electrochemical characteristics.

Acknowledgements

This study was supported by NRF(R13-2008-000-00000-0).

References

[1] Semlitsch MF, Weber H, Streicher RM and Schon R. Joint replacement components made of hot-forged and surface treated Ti–6Al–7Nb alloy. *Biomaterials* 1992;**13**: 781.

[2] Okazaki Y, Rao S, Asao S, Tateishi T, Katsuda S and Furuki Y. Effect of Ti, Al and V concentration of the relative growth ratio of Bio-Cells. *J. Japan Inst Metals* 1996; **9**: 890.

[3] Zhou YL, Niinomi M, Akahori T, Fukui H and Toda H. Corrosion resistance and biocompatibility of Ti-Ta alloys for biomedical applications. *Materials Sci and Eng A* 2005; **398**: 28.

[4] Nosova GI. *Phases transformation in titanium alloys*. Nauka, Moscow (1968).

[5] Buser D, Nydegger T, Oxaland T. Interface shear strength of titanium implants with sandblasted and acid-etched surface, A biomechanical study in the maxialla of miniature pigs. *J Biomed Mater Res* 1999; **45**:75.

[6] Choe HC. Nanotube and micropore of Ti alloy systems for biocompatibility. *Materials Science Forum* 2010;**654**:2061-64

[7] Choe HC, Kim WG, Jeong YH. Surface characteristics of HA coated Ti-30Ta-xZr and Ti-30Nb-xZr alloys after nanotube formation. *Surf. & Coat. Tech* 2010;**205**:S305-11.

[8] Linez-Bataillon P, Monchau F, Bigerelle M, Hildebrand HF. In vitro MC3T3 osteoblast adhesion with respect to surface roughness of Ti6Al4V substrates. *Biomolecular Engineering* 2002;**19**:133.

[9] Anselme K, Linez P, Bigerelle M. The relative influence of the topography and chemistry of Ti6Al4V surfaces on osteoblastic cell behaviour. *Biomaterials* 2000;**21**:1567.

[10] Von Recum AF and van Kooten TG. The influence of micro-topography on cellular response and the implications for silicon implant. *J. Biomater. Sci. Polymer. ED.* 1995; **7**:181.

[11] Schwartz Z, Kieswetter K, Dean DD, Boyan BD. Underlying mechanism at the bone-surface interface during regeneration. *J. Periodontal Res* 1997; **32-1**:166.

[12] Gonzalez JEG, Mirza-Rosca JC. Study of the corrosion behavior of titanium and some of its alloys for biomedical and dental implant applications. *Journal of Electroanalytical Chemistry* 1999; **471**:109.

[13] Banerjee R, Nag S, Stechschulte J and Fraser HL. Strengthening mechanisms in Ti-Nb-Zr-Ta and Ti-Mo-Zr-Fe orthopedic alloys. *Biomaterials* 2004;**25**: 3413.